Gene Sequence Structure *

211 bp   Sequence Deleted   355 bp
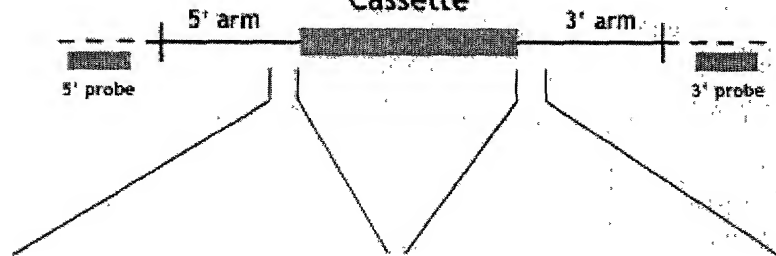
Size of full-length cDNA: 2486 bp

Targeting Vector* (genomic sequence)

LacZ-Neo Cassette

5' arm

3' arm

5' probe

3' probe

Arm Length:
5': 2.5 kb
3': 1 kb

———— Targeting Vector
– – – – Endogenous Locus

* Not drawn to scale

5'>CATGCATACATGGGTGCACGT
GCGTGCGTGCAAACACCCACACAC
AGATAGATAAATGTAAAAAAATAC
TTAGGCAATGCTGTTTTTTTTAAG
CTTGGGAAATTAATATTTTTGAAC
ACTGCTGATGTGGGATTTTAAGTT
ACAAATGTTAAACTTGTTCTCCTT
AGTGACTTTTGGGATGGTCAATCC
ACATATGTACT<3'
(SEQ ID NO:3)

5'>GTATGGCAACAAACAGCTGTA
CAGTGTGAAGAACAGCAGCCGCAT
CTACTACGAGAACGTTCTTCTCGG
CATCCCCAGAGTGCGGCAACTGCG
AGTCCGAAACAACACTTGCAAGGT
CTACCCAGCTTTCCAGTCCCTGGT
CAGCGACTGCTACAGCAAGTACAC
AGTGGAAAACGAAGACTTCTCTGA
TTTTGGCCTCA<3'
(SEQ ID NO:4)

FIGURE 4

GGCAGAACGGGCATAGTGGAACGGACCGTGTGGTATGCTCCGCGATGTCTGAGGCGACTTGGTG
GTACCGAGGAGGGACTTCAAAACATGACCTGCATTACAGAAGGGAAGCGGAGGTTAACACCACA
CTCGAGGAGTTGTTACTCTATTTTATTTTCTTAATAAATCTATGCATATTGACTTTTGGGATGG
TCAATCCACATATGTACTATTTAAACAAAGTTATGTCGTCTCTGTTTGTGGACACTTCTCTACC
TGATGATGAAGAAGCAGCTTTAGGTCCATTCGGAGCATAACTGAGTTTTGGAAGTTCATGGAA
GGACCCCTCATCGACGGCTTGTACTGGGACTCGTGGTATGGCACCAAACAGCTGTACAGTGTGA
AGAACAGCAGCCGCATCTACTACGAGAACGTTCTTCTCGGCATCCCCAGAGTGCGGCAACTGCG
AGTCCGAAACAACACTTGCAAGGTCTACCCAGCTTTCCAGTCCCTGGTCAGCGACTGCTACAGC
AAGTACACAGTGGAAAACGAAGACTTCTCTGATTTTGGCCTCAAACGCAATCCAGAATGGACGC
ACACGCCTTCTTCCCGCACTGCCCCATGGCACTGGGGGTTTGTTGGCGTATACCGAGATGGAGG
ATATATAGTCACGTTATCAAAATCAAAATCTGAAACCAAAGCCAAATTTGTTGACCTTCGACTG
AACAACTGGATTAGCAGAGGCACCAGGGCTGTTTTATTGATTTCTCCCTGTACAATGCTAATG
TCAACCTGTTTTGCATCATCAGGCTGCTGGCAGAGTTCCCTGCGACGGGTGGGCTCCTCACCTC
CTGGCAGTTCTACTCTGTGAAGCTCCTCAGATACGTCTCCTACTACGACTACTTCATTGCCTCC
TGTGAAGTCATATTTTGTATTTTTCTCTTTGTCTTCATAATACAAGAACTGAGGAAAGTGAACG
AGTTTAAGTCTGCCTATTTCAGAAGTGTCTGGAACTGGCTGGAGATGCTGCTCCTGCTGCTCTG
TTTTCTCGCCGTGTCTTTCTATGCATACTGTAACATGCAGAGCTTTCTCTTGCTTGGACAGCTG
CTGAAAAACACTGACAGCTATCCCGACTTTTACTTCCTTGCATACTGGCACATTTACTATAACA
ACGTAATTGCTATCACTATCTTCTTTGCATGGATAAAGATATTCAAGTTCATAAGCTTCAATGA
GACAATGTCGCAGCTGTCATCAACACTCTCCCGCTGCATGAAGGACATCGTGGGGTTCGCCATC
ATGTTCTTCATCATCTTCTCTGCTTATGCCCAGTTGGGATTTCTGGTTTTTGGGTCACAGGTTG
ATGATTTTTCAACTTTTCAAAATTCCATATTTGCACAATTTCGAATTGTCCTCGGGGACTTTAA
CTTTGCTGGCATCCAGCAGGCCAACTGGATCTTGGGGCCCATCTACTTCATCACGTTCATCTTC
TTTGTGTTCTTTGTGCTCCTGAACATGTTCTTGGCAATAATTAATGACACCTATTCTGAAGTTA
AGGCTGATTATTCAATAGGCAGAAGACCAGATTTTGAACTTGGTAAAATAATTCAAAAGAGTTG
CTTTAATGTTCTCGAGAAACTCAGACTCAAGAAAGCTCAAGCTAAAGAAGAAAAGAAAATGCAA
ACCACTGACTTGGCCCAGAGAGCCAGAAGAGAAGGCTTTGATGAAAGTGAGATCCAAGAGGCAG
AGCAGATGAAAGATGGAAGGAAAGGCTTGAAAAAAAGTATTATTCTACAGAAATTCAAGACGA
TTATCAGCCTGTCACTCAGCAAGAATTCCGAGAGCTCTTTTTATACGCGGTGGAGCTTGAGAAG
GAATTACACTATGTCAGTTTAAAACTGAACCAACTGATGAGAAAGCTGCACTAGCAGGCTGACA
AGTGGAGTCATTTTATAAGAAAGGCAACCGAAGAATTTCATTCAGTATGAAGATTTTTTCCTC
TCAATTTTCTTCAGTAATGCAAAAGAAAAACCAAAAGTAACCAGAAGTGCTTTTTATTTCAAA
GTTCTTGAAGTAAAAGAGTAAAACTCTTGTCCTTTGCTAACAGCCGTGTCTGCAGTAAAACAAT
GAAGGAGCCTGCGTGTTTCCTAAGTGTGGAGAGGATCTGCGGGAATGTGGAACAGCTTTCCTTG
CCTACTGGAACCACAAACAAGCACACAATGGGACTCTCTGAGTGCCTGACAAAGTGAACGCAAG
TACAGCCAAGCACACATGGTGAACTGTCAGGGAACACAAGCACTTTATGGCGTCAACTTTCAAG
GAACATATTTTATATGGATTTTGAAGAGTCTTGTTTGCTGATAAGAACTTCAAGAAGTCTAAGC
TTGGCTTTGATTCTCTTGTATTCCTTATATTCCTCAAGCACCGGAACACGATCCTCCTTCTGGG
CATTCCTAGGGAAGATAAAACTCTGTAAAGCAAAAAAAGAAAAAAAAAAAAAAA (SEQ ID
NO:1)

FIGURE 1

MSEATWWYRGGTSKHDLHYRREAEVNTTLEELLLYFIFLINLCILTFGMVNPHMYYLNKVMSSL
FVDTSLPDDERSSFRSIRSITEFWKFMEGPLIDGLYWDSWYGTKQLYSVKNSSRIYYENVLLGI
PRVRQLRVRNNTCKVYPAFQSLVSDCYSKYTVENEDFSDFGLKRNPEWTHTPSSRTAPWHWGFV
GVYRDGGYIVTLSKSKSETKAKFVDLRLNNWISRGTRAVFIDFSLYNANVNLFCIIRLLAEFPA
TGGLLTSWQFYSVKLLRYVSYYDYFIASCEVIFCIFLFVFIIQELRKVNEFKSAYFRSVWNWLE
MLLLLLCFLAVSFYAYCNMQSFLLLGQLLKNTDSYPDFYFLAYWHIYYNNVIAITIFFAWIKIF
KFISFNETMSQLSSTLSRCMKDIVGFAIMFFIIFSAYAQLGFLVFGSQVDDFSTFQNSIFAQFR
IVLGDFNFAGIQQANWILGPIYFITFIFFVFFVLLNMFLAIINDTYSEVKADYSIGRRPDFELG
KIIQKSCFNVLEKLRLKKAQAKEEKKMQTTDLAQRARREGFDESEIQEAEQMKRWKERLEKKYY
STEIQDDYQPVTQQEFRELFLYAVELEKELHYVSLKLNQLMRKLH (SEQ ID NO:2)

FIGURE 2

underlined = deleted in targeting construct

BOLD = sequence flanking Neo insert in targeting construct

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GGCAGAACGGGCATAGTGGAACGGACCGTGTGGTATGCTCCGCGATGTCTGAGGCGACTT
GGTGGTACCGAGGAGGGACTTCAAAACATGACCTGCATTACAGAAGGGAAGCGGAGGTTA
ACACCACACTCGAGGAGTTGTTACTCTATTTTATTTTCTTAATAAATCTATGCATATTGA
CTTTTGGGATGGTCAATCCACATATGTACTATTTAAACAAAGTTATGTCGTCTCTGTTTG
TGGACACTTCTCTACCTGATGATGAAAGAAGCAGCTTTAGGTCCATTCGGAGCATAACTG
AGTTTTGGAAGTTCATGGAAGGACCCCTCATCGACGGCTTGTACTGGGACTCGTGGTATG
GCACCAAACAGCTGTACAGTGTGAAGAACAGCAGCCGCATCTACTACGAGAACGTTCTTC
TCGGCATCCCCAGAGTGCGGCAACTGCGAGTCCGAAACAACACTTGCAAGGTCTACCCAG
CTTTCCAGTCCCTGGTCAGCGACTGCTACAGCAAGTACACAGTGGAAAACGAAGACTTCT
CTGATTTTGGCCTCAAACGCAATCCAGAATGGACGCACACGCCTTCTTCCCGCACTGCCC
CATGGCACTGGGGGTTTGTTGGCGTATACCGAGATGGAGGATATATAGTCACGTTATCAA
AATCAAAATCTGAAACCAAAGCCAAATTTGTTGACCTTCGACTGAACAACTGGATTAGCA
GAGGCACCAGGGCTGTTTTTATTGATTTCTCCCTGTACAATGCTAATGTCAACCTGTTTT
GCATCATCAGGCTGCTGGCAGAGTTCCCTGCGACGGGTGGGCTCCTCACCTCCTGGCAGT
TCTACTCTGTGAAGCTCCTCAGATACGTCTCCTACTACGACTACTTCATTGCCTCCTGTG
AAGTCATATTTTGTATTTTTCTCTTTGTCTTCATAATACAAGAACTGAGGAAAGTGAACG
AGTTTAAGTCTGCCTATTTCAGAAGTGTCTGGAACTGGCTGGAGATGCTGCTCCTGCTGC
TCTGTTTTCTCGCCGTGTCTTTCTATGCATACTGTAACATGCAGAGCTTTCTCTTGCTTG
GACAGCTGCTGAAAAACACTGACAGCTATCCCGACTTTTACTTCCTTGCATACTGGCACA
TTTACTATAACAACGTAATTGCTATCACTATCTTCTTTGCATGGATAAAGATATTCAAGT
TCATAAGCTTCAATGAGACAATGTCGCAGCTGTCATCAACACTCTCCCGCTGCATGAAGG
ACATCGTGGGGTTCGCCATCATGTTCTTCATCATCTTCTCTGCTTATGCCCAGTTGGGAT
TTCTGGTTTTTGGGTCACAGGTTGATGATTTTTCAACTTTTCAAAATTCCATATTTGCAC
AATTTCGAATTGTCCTCGGGGACTTTAACTTTGCTGGCATCCAGCAGGCCAACTGGATCT
TGGGGCCCATCTACTTCATCACGTTCATCTTCTTTGTGTTCTTTGTGCTCCTGAACATGT
TCTTGGCAATAATTAATGACACCTATTCTGAAGTTAAGGCTGATTATTCAATAGGCAGAA
GACCAGATTTTGAACTTGGTAAAATAATTCAAAAGAGTTGCTTTAATGTTCTCGAGAAAC
TCAGACTCAAGAAAGCTCAAGCTAAAGAAGAAAGAAAATGCAAACCACTGACTTGGCCC
AGAGAGCCAGAAGAGAAGGCTTTGATGAAAGTGAGATCCAAGAGGCAGAGCAGATGAAAA
GATGGAAGGAAAGGCTTGAAAAAAAGTATTATTCTACAGAAATTCAAGACGATTATCAGC
CTGTCACTCAGCAAGAATTCCGAGAGCTCTTTTTATACGCGGTGGAGCTTGAGAAGGAAT
TACACTATGTCAGTTTAAAACTGAACCAACTGATGAGAAAGCTGCACTAGCAGGCTGACA
AGTGGAGTCATTTTATAAGAAAAGGCAACCGAAGAATTTCATTCAGTATGAAGATTTTTT
CCTCTCAATTTTCTTCAGTAATGCAAAAGAAAAACCAAAAAGTAACCAGAAGTGCTTTTT
ATTTCAAAGTTCTTGAAGTAAAAGAGTAAAACTCTTGTCCTTTGCTAACAGCCGTGTCTG
CAGTAAAACAATGAAGGAGCCTGCGTGTTCCTAAGTGTGGAGAGGATCTGCGGGAATGT
GGAACAGCTTTCCTTGCCTACTGGAACCACAAACAAGCACACAATGGGACTCTCTGAGTG
CCTGACAAAGTGAACGCAAGTACAGCCAAGCACACATGGTGAACTGTCAGGGAACACAAG
CACTTTATGGCGTCAACTTTCAAGGAACATATTTTATATGGATTTTGAAGAGTCTTGTTT
GCTGATAAGAACTTCAAGAAGTCTAAGCTTGGCTTTGATTCTCTTGTATTCCTTATATTC
CTCAAGCACCGGAACACGATCCTCCTTCTGGGCATTCCTAGGGAAGATAAAACTCTGTAA
AGCAAAAAAAGAAAAAAAAAAAAA
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